Uric Acid in Parkinson's disease

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Academic Dissertation

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Abstrakti

Parkinsonin-tauti on yleisin liikehäiriösairaus, jonka esiintyvyys yli 60 vuoden ikäisessä väestössä on noin 1%. Parkinsonin-taudin syy on tuntematon mutta useita elämäntapoihin ja ympäristötekijöihin liittyviä riskitekijöitä tunnetaan. Todennäköisesti myös perinnölliset tekijät vaikuttavat sporadisenkin Parkinsonin-taudin riskiin.

Korkean plasman uraatti-pitoisuuden on havaittu vähentävän riskiä sairastua Parkinsonin-tautiin ja myös hidastavan motoristen oireiden etenemistä taudin puhjettua. Väitöskirjan hypoteesina oli että Parkinsonin-tautia sairastavilla olisi matalampi plasman uraatti-pitoisuus kuin terveillä puoliso-verrokeilla ja että matala uraatti-pitoisuus altistaisi potilaita neurokognitiivisille ja neurofysiologisille muutoksille seuranta-aikana.

Plasman uraatti mitattiin 40 potilaalta ja 28 terveeltä puoliso-verrokilta. Potilaille tehtiin neuropsykologinen tutkimus, joka toistettiin 3 vuoden seurannan jälkeen. 18 potilaalta ja 24 verrokilta rekisteröitiin aivosähkökäyrä, jonka yhteydessä mitattiin kuulo-herätevasteita. Tämän neurofysiologisen tutkimuksen tarkoituksena oli selvittää eroavaisivatko potilaiden ja verrokkien herätevasteet toisistaan ja vaikuttaisivatko Parkinsonin-taudin aiheuttamat neurokognitiiviset muutokset herätevasteiden havaitsemiseen.

Tutkimuksessa osoitettiin että Parkinsonin-tautia sairastavilla on merkittävästi matalammat plasman uraatti-pitoisuudet kuin verrokeilla. Potilailla matala plasman ja vuorokausivirtsan uraatti assosioituivat alentuneeseen tarkkaavuuteen ja toiminnanohjaukseen ja vaikeuksiin näönvaraisessa hahmottamisessa.

Kolmen vuoden seuranta-aikana potilaiden kognitiivisessa tilanteessa ei tapahtunut merkittäviä muutoksia. Kognitio ei myöskään vaikuttanut assosioituvan uraatti-tasoihin enää seuranta-ajan lopussa.

Herätevaste-tutkimuksessa havaittiin, että Parkinsonin-tautia sairastavilla N100-vasteen latenssi oli pidempi ja vasteen habituaatio heikompi kuin verrokeilla. Nämä muutokset korreloivat neuropsykologisessa tutkimuksessa havaittuun näönvaraisen työmuistin heikentymiseen mutta eivät plasman tai virtsan uraatti-pitoisuuteen.

Abstract

Parkinson's disease (PD) is the most common movement disorder, affecting approximately 1% of the population aged over 60 years. According to current knowledge, there are both genetic and environmental risk factors for this disease. High plasma uric acid level has been shown to reduce the risk of PD and also to decelerate the progression of motor symptoms in patients with PD.

In this study, 40 PD patients and 28 spouse controls were recruited to examine whether patients have low plasma and daily urine uric acid levels relative to the controls. Also neuropsychological testing was performed on patients both at baseline and after three years of follow-up to determine whether cognition is associated with the uric acid levels and whether low levels of uric acid at baseline predict future cognitive decline. An electroencephalogram with evoked response potentials (EEG-ERP) was given to a subsample of patients and controls to examine neurophysiological differences between the groups and to investigate whether cognitive alterations are reflected in patients' EEG-ERP measurements.

PD patients had lower levels of plasma uric acid than controls. Low plasma and urine uric acid levels were associated with poor achievement in neuropsychological tests measuring visuospatial and visuoconstructive abilities, sustaining attention and executive control. After three years of follow-up, only minor cognitive decline was noted in patients. Cognition did not seem to be associated with past or present uric acid levels. On the other hand, the stability of the patient sample made it difficult to assess the effect of uric acid on the prognosis of cognitive decline in PD.

The EEG-ERP study revealed that PD patients had longer latency and poorer habituation of the evoked response potential N100 than healthy controls. In the patient group, the neurophysiological changes were associated with poor achievement in a neuropsychological test measuring visual working memory, but not with uric acid levels.

Introduction

Parkinson's disease (PD) is a complex neurodegenerative disorder with multiple symptoms from a wide area of the central nervous system.

The common age of onset is above 50 years, but especially the rare genetic forms and also sporadic PD may begin earlier. The motor symptoms of PD comprise slowness of movement, muscle rigidity and resting tremor. This classic triad, having regularly an asymmetrical onset, forms the cornerstone of the diagnosis, which is still mainly based on clinical examination. According to clinico-pathological studies, the accuracy of the diagnosis is approximately 75%[1]. Computed tomography (CT) and magnetic resonance imaging (MRI) are useful only to rule out other causes such as vascular lesions and tumours.

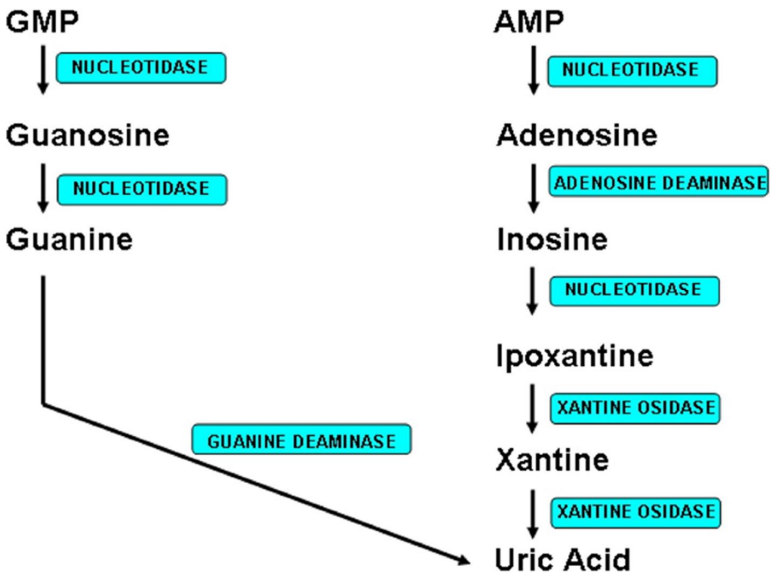
The motor symptoms of PD were long considered to constitute the major burden of the disease. However, the non-motor symptoms often have an enormous impact on the quality of life. Diminished or absent sense of smell, REM sleep behaviour disorder and constipation are early signs of PD and may precede the occurrence of motor symptoms by years[2]. Later on, facial expressions are diminished, the voice becomes forceless and dysarthria may develop, with devastating social consequences. The architecture of sleep is often fragmented, forcing patients to wake up several times during the night or to wake up early without being able to fall back asleep. Bladder frequency typically increases and nocturia may add to sleep disturbance. Orthostatic hypotension and balance problems produce falls and hinder the patients' willingness to move, thus adding to disability[3].

The most devastating consequence is cognitive decline and ensuing Parkinson's disease dementia (PDD), which cause a gradual loss of independence and usually a substantial caregiver burden. The cognitive symptoms have insidious onset and the episodic memory often remains spared for a long time. The common first symptoms are slowness of thought, word finding difficulty and visual and executive dysfunction. Working memory is often disturbed, but more profound amnestic syndrome usually develops later[4-6].

There are currently numerous pharmacological and neurosurgical treatments available for the motor symptoms, but few for cognitive decline. Dementia also limits the use of PD medicines to treat the motor symptoms, as hallucinations and even psychoses may develop as side effects. Also neurosurgical interventions are not possible with PDD. In view of these limitations, it is easy to see why identification of treatable risk factors for cognitive decline in PD is

imperative. Emerging treatment options also prompt the need for diagnostic tools to detect cognitive decline and dementia in PD.

In humans, uric acid is the end product of purine metabolism[7]. The level of uric acid in the plasma/serum varies according to body mass index (BMI) and genetic and dietary factors, animal proteins contributing significantly to the level of purines available to uric acid synthesis[8]. Below is a picture of the metabolic pathway of uric acid. In other mammals, uric acid is further degraded to allantoin, but humans have lost the uricase enzyme during evolution. This may have even contributed to the long life span of humans since uric acid's antioxidative qualities may function as a preservative that makes tissues more tolerant to oxidative stress and ageing.



GMP=guanosine monophosphate

AMP=adenosine monophosphate

Uric acid is a powerful antioxidant and iron binding protein, making it an intriguing research subject in neurological diseases such as PD, where oxidative stress and locally increased iron level seem to play an active part. In epidemiological studies, a high level of serum uric acid has been found to reduce the risk of PD. In early PD, uric acid has been observed to attenuate the progression of both motor symptoms and nigral cell damage, with uric acid thus being the first recognized molecule to slow disease progression in PD[9].

Little data exist on serum uric acid levels in PD patients or its effect on non-motor symptoms such as cognitive decline. Given the lack of treatments to alter the disease course and the high prevalence of cognitive symptoms in PD, this thesis aimed to investigate whether patients with PD have low plasma uric acid levels and whether uric acid contributes to cognition in PD. In the future, increasing the plasma uric acid level might be a therapeutic option to slow the progression of both motor and cognitive symptoms in PD.

Review of the literature

Key epidemiological findings

In 1981, Ames et al. presented a novel hypothesis of uric acid, arguing that it would provide an antioxidant defence in humans against oxidant- and radical-caused ageing and cancer[10]. As oxidative stress has long been considered one of the mechanisms of cellular damage in PD, it was plausible to assume that a high serum uric acid level would protect from PD. To test this hypothesis, Davis et al. conducted a prospective study among 8006 men of Japanese or Okinawan ancestry, who participated in the Honolulu Heart Program. The principal aim of the Honolulu Heart Program was to examine whether Westernization would have an impact on cardiovascular morbidity and mortality. As uric acid is a risk factor for cardiovascular disease, it was also measured at baseline. The cohort was prospectively followed for 30 years, during which time 92 new cases of PD were observed. Only the diagnoses made by a neurologist or a neurosurgeon were included in the study. Men with above median serum uric acid had a 30% reduction in the risk of PD[11]. This finding gained little interest in the research community until de Lau et al. reported similar findings from a large, population-based cohort from the Netherlands in 2005. The study population included 4695 participants, both men and women, aged 55 years or older. Subjects with PD or signs of parkinsonism at baseline were excluded. Serum uric acid was measured at baseline and the cohort was repeatedly screened for signs of PD in a standardized fashion for over 9 years. During follow-up 68 new cases of PD were identified. Cox proportional hazards model showed a significant reduction in the risk of PD with increasing serum uric acid concentration. The risk reduction seemed to be equally strong in both men and women[12].

There are also patient populations suffering from long-term hyperuricemia, a state of abnormally high serum uric acid level, causing gouty arthritis. Gout is a metabolic disorder that is characterized by increased serum uric acid level and deposition of urate crystals in joints and kidneys, causing acute painful arthritis and kidney stones. One would expect that the long-term hyperuricemia in gout would lead to a reduced risk of PD. There are several studies examining this association, but the findings have been contradictory. While some studies have shown an approximately 30% reduction in the risk of PD[13-15], others have found no association[16,17]. Most studies have been registry-based and have used the prescription of anti-gout medication as a proxy for the diagnosis of gout. Even when a diagnosis of gout was mandatory in the study protocol, the diagnostic criteria applied had not been explicitly defined. This variation in the case ascertainment may explain some of the contradictory findings. As with uric acid, some studies have found gout protective of PD in men only, which may also be a source of bias in these studies.

Lifestyle factors and risk of PD, a link to uric acid?

Milk and dairy products

Consumption of milk and dairy products has been found to increase the risk of PD [18]. This association has been observed in both men and women. Drinking milk in adulthood is common in Finland, and a positive association between milk and PD also emerged in a Finnish study by Sääksjärvi et al.[19]

At first, it was speculated that milk and dairy products might contain contaminants, such as pesticides, from cows' feed. This hypothesis was strengthened by a recent study measuring the neuronal density of substantia nigra in healthy postmortem brains[20]. An intake of milk exceeding 16 oz (263 ml) daily was associated with a 40% reduction in neuron density. The reason for this remained obscure, but there were also traces of heptachlor epoxide in the brain slices. This pesticide was previously found in milk in the area where the study subjects lived and where the milk was produced. Exposure to pesticides is a known risk factor of PD, but increased risk in association with heptachlor epoxide has not been shown.

Our study group has presented an alternative hypothesis of an association between milk and serum uric acid levels. Milk is known to decrease serum uric acid levels, probably by increasing the excretion of uric acid in the kidneys. Daily consumption of milk and dairy products has also been shown to decrease serum uric acid levels in a large epidemiological study including 14 809 participants[21]. Regular intake of milk and dairy products might therefore decrease the serum uric acid levels and predispose to PD. This hypothesis was also considered more likely in a recent review[22].

Weight loss

Weight loss is also associated with low levels of serum uric acid, and gradual loss of weight increases the risk of PD. This was found in the Harvard Alumni Health Study, comprising 12 228 men, 88.4% of whom had adhered to the study until the end of follow-up. Eventually, 106 men were diagnosed with PD. Men losing 0.5 units of body mass index per decade had a 2.6 multivariate relative risk of PD[23]. Unexplained weight loss is common in PD, and this finding may reflect the long prodromal period of PD[24].

Caffeine

The role of caffeine in PD has long puzzled neurologists. Whether caffeine protects from PD was prospectively examined in the Honolulu Heart Program described earlier. Coffee drinkers had a significantly lower incidence of PD than non-drinkers. For those who did not consume coffee at all, the risk for PD was

2-3 times higher[25]. This finding has since been replicated by others, and there is also a Finnish study on the subject. This population-based study included 6710 Finns, with an extensive follow-up of 22 years. During this time 101 incident cases of PD occurred. After adjusting for various potential confounders, the heavy coffee drinkers were found to have a 0.26 relative risk of PD compared with non-drinkers[26].

The most likely explanation for this association is caffeine's function as an adenosine A2a-receptor antagonist[27]. Adenosine A2a-receptors and dopamine D2-receptors interact in the substantia nigra, and blocking of the adenosine A2a-receptors increases the level of available dopamine. This provides symptomatic relief in PD. In the cellular models of PD, caffeine has prevented neuronal death induced by MTPT, a finding that is also mediated by the adenosine A2a-receptor[28]. Apparently, adenosine receptor antagonism reduces glutamate-mediated toxicity in the substantia nigra and reduces the inflammatory actions of glial cells, which also express these receptors.

Our group has proposed an additional mechanism of caffeine's neuroprotective action. Molecularly, caffeine is a purine alkaloid that metabolizes in the body to form theobromine and xanthine, both having strong antioxidant capacity[29] and structural similarity to uric acid. Xanthine also metabolizes further to produce uric acid. We have therefore suggested that regular caffeine consumption would reflect years of self-administered antioxidant intake.

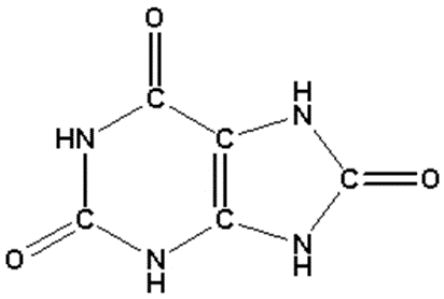
Tea

While the protective effect of coffee has been attributed to caffeine, there is another component in tea that seems to provide an equal benefit. Drinking tea has been shown to reduce the risk of PD, and this risk reduction was first assumed to be caused by caffeine in tea. In the Singapore Chinese Health Study, the authors were able to show that black tea was associated with reduced risk of PD and that the risk reduction was independent of caffeine[30]. In this study, the oestrogen levels were highest in the drinkers of black tea. As both exogenous and endogenous oestrogens seem to reduce the risk of PD, the authors suggested that some ingredient of tea might operate through an oestrogen-mediated mechanism. There is also evidence of antioxidant capacity in both green and black tea extracts, though the biological significance in humans is uncertain.

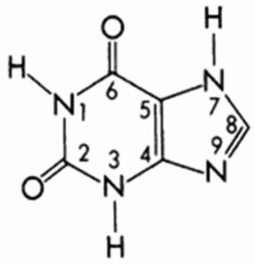
In addition to the other antioxidants, tea is known to contain small amounts of methylxanthine, known as theophylline in pharmacology. Theophylline resembles caffeine in that both are purine alkaloids and adenosine receptor antagonists. Xanthine is also a precursor of uric acid, and the methylated form

may either mimic the action of uric acid or increase its serum levels, although this has not been shown. The protective effect of tea may therefore be mediated by a mechanism similar to that of uric acid.

The structural similarity of uric acid, xanthine, caffeine, theophylline and theobromine can be seen in the pictures showing their respective molecular structures.



Uric Acid



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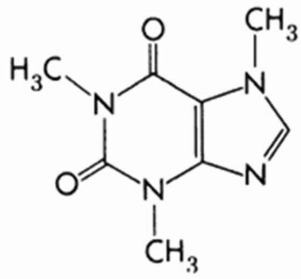

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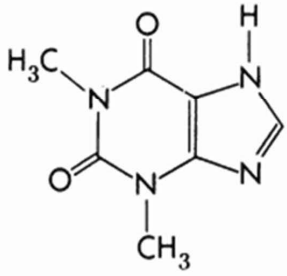

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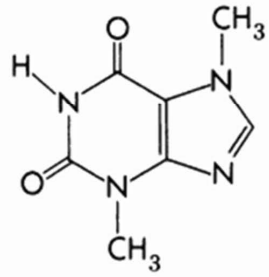


THEOBROMINE

Other factors related to risk of PD

In addition to the lifestyle factors presented above, there are other known modifiers of the risk of PD.

Smoking tobacco has been found to decrease the risk of PD by up to 70% with increased duration of smoking [31,32]. It was previously suspected that the association between smoking and PD risk might be explained by certain personality traits of people prone to have PD. These individuals would be risk-aversive and less sensation-seeking, and perhaps their dopamine metabolism would be altered in a way that would make them less prone to addictions. In light of the accumulating epidemiological evidence, this theory has been abandoned. The protective effect of tobacco is most likely mediated by nicotine, and animal studies also favour this hypothesis[33]. The effect of some other, yet unrecognized substances in tobacco cannot be ruled out. A randomized trial using a transdermal nicotine patch in patients with early PD is ongoing (NCT01560754).

Physical activity during adolescence and adulthood carries a decreased risk of PD in later life[34,35]. The explanation for this epidemiological finding is not known, but physical activity has been suggested to protect neurons by regulating dopamine turnover[36], increasing the release of neurotrophic factors[37] and increasing serum uric acid[38].

Regular use of non-steroidal anti-inflammatory drugs, especially ibuprofen, reduces the risk of PD[39]. This finding is probably explained by the anti-inflammatory action that supresses the microglia's inflammatory response.

Regular use of calcium-channel blockers that are commonly used to treat hypertension also reduces the risk of PD[40]. The mechanism of the possible neuroprotective action of these drugs is probably a blockade of calcium channel-mediated metabolic stress on mitochondria of the dopaminergic neurons. Because of this scientifically acceptable explanation and a known safety profile, isradipine is currently being investigated in treatment of early PD (NCT02168842).

Alcohol also offers a slight decrease in the risk of PD when comparing drinkers and non-drinkers. This may also be explained by the uric acid-elevating qualities of alcohol[41].

Exposure to pesticides is related to increased risk of PD[42]. The evidence for this comes from agricultural studies, where farm work explains long-term exposure. Although no specific compound has been recognized as a major risk factor, the

ones that cause a defect of mitochondrial complex I or oxidative stress seem the likeliest candidates.

Genetic factors

The serum uric acid levels depend on weight and diet, but there is also genetic variability. Gonzalez-Aramburu et al. conducted a large study on 1061 PD patients and 754 normal controls. They analysed polymorphisms in 9 genes known to regulate serum uric acid levels and found that having 9 or more polymorphisms that decreased the level of uric acid increased the risk of PD 1.5-fold. This association was found in both men and women [43]. In another study recruiting early-stage PD patients from both the DATATOP and PRECEPT study populations, the researchers analysed single-nucleotide polymorphisms (SNPs) in the urate transporter SLC2A9 gene. This is the strongest known genetic regulator of serum uric levels. The patients with SNPs producing low serum uric acid levels had a greater risk of progression to disability requiring levodopa therapy. They also had significantly lower levels of serum uric acid than the patients without these SNPs [44].

Pathogenesis of PD

To find explanations for the view that uric acid might be neuroprotective in PD, one must first look at the pathogenic mechanisms of the disease.

The neuropathology of PD involves the appearance of inclusion bodies in both cellular processes and soma of neurons, called Lewy neurites and Lewy bodies, respectively[45]. The Lewy neurites and Lewy bodies are composed of fibrillar, phosphorylated alpha-synuclein, which has gone through a conformational change into a beta-sheet, lost its membrane binding capacity and aggregated. Normally, alpha-synuclein is present in various cell types and tissues. In the brain, it is expressed in the presynaptic terminals of neurons. Its physiological functions are not fully understood, but it most likely participates in regulating vesicular release and synaptic plasticity as well as mitochondrial complex 1 activity. Alpha-synuclein is able to adopt different conformations to guide its actions. This plasticity makes it vulnerable to changes in the cellular environment, which may induce the formation of beta-sheet structure and ensuing alpha-synuclein aggregation[46].

Although Lewy bodies are the pathological hallmark of PD, it is not known whether they are active participants in the pathological process of PD or whether they are formed to sequester toxic, aggregated alpha-synuclein from disturbing cellular homeostasis.

There are rare genetic forms of PD with overexpression of or mutations in the alpha-synuclein gene SNCA. The clinical picture is often earlier onset and more aggressive, but otherwise the symptoms and neuropathological findings bear a close resemblance to the sporadic disease. The overexpression of alpha-synuclein seems therefore sufficient to produce its aggregation and neurodegeneration of dopaminergic neurons[47]. The mutations in the SNCA-gene on the other hand stabilize beta-sheet structure and also promote aggregation. In light of this, it seems likely that at least alpha-synuclein is an active player in the pathogenesis of PD[48].

The familial forms of PD are rare, and traditionally, the influence of genes has been considered very low in sporadic PD. In twin studies, the hereditability of PD has been low and concordance even in monozygotic twins has been no more than 15%[49]. In Finland, Kuopio et al. studied familial occurrence of PD in a community-based sample. The odds ratio of having a first-degree relative with PD was 2.7 in patients compared with controls. The authors stated that this finding may not be explained solely by genetic factors, but that shared environmental risk factors may also contribute[50].

The advent of genome-wide association (GWA) techniques may change our views about the importance of genes in PD. GWA studies have so far revealed that although there are no high-risk alleles for PD, there are certain risk loci at

the genes encoding both alpha-synuclein and microtubule-associated protein tau (MAPT) as well as in the genes regulating membrane trafficking, endocytosis and proteolysis. These findings point to there being some genetic background in PD, although the genetic risk at the individual level is low and may require a combination of several risk alleles as well as the presence of environmental or lifestyle risk factors. The GWA studies also give important clues to the pathogenesis. While overexpression of alpha-synuclein may be sufficient to induce its oligomerization and aggregation, dysfunction of the protein degradation pathways, such as proteolysis and lysosomal clearance, also increase the level of alpha-synuclein[51].

In humans, according to Braak[52], the accumulation of alpha-synuclein starts in the lower brain stem, especially in the dorsal motor nucleus of the vagal nerve (DMV) and the olfactory system. From the lower brain stem, the alpha-synucleinopathy then ascends from the DMV to the raphe nucleus, the reticular formation and the locus coruleus. Eventually, the disease process reaches the neuromelanin-containing neurons of the substantia nigra and the patient enters a symptomatic stage in which the first motor symptoms appear. The disease does not remain confined to the substantia nigra, but ascends further and eventually involves the whole brain. At the later stages, the Lewy bodies are also seen in the neocortex. Neural cell death is observed especially in the substantia nigra, where the loss of pigmented, neuromelanin-containing dopaminergic neurons leads to motor symptoms. Substantial neural cell death also occurs in the nucleus basalis of Meynert, locus coruleus and raphe nucleus.

While the reason for the selective death of the dopaminergic neurons in PD is unclear, multiple mechanisms of disease have been recognized. In addition to alpha-synuclein aggregation, increased oxidative stress, activation of microglia and ensuing neuroinflammation, dysfunction of mitochondrial complex 1, accumulation of iron and dysfunction of protein degradation pathways all contribute to the neurodegeneration in PD.

Microglia are the brain's resident macrophages that sense changes in the cellular environment and respond to them in an attempt to maintain cellular homeostasis. The microglia also try to clear excess alpha-synuclein from the extracellular space, but, due to alpha-synuclein toxicity, this leads to proinflammatory changes and increased production of reactive oxygen species (ROS) and increased oxidative stress[53]. Binding of soluble alpha-synuclein to microglial membrane proteins also leads to activation of inflammatory pathways that eventually induce a proinflammatory response also in the astrocytes, further increasing the oxidative burden.

Alpha-synuclein affects mitochondrial membrane trafficking by inhibiting protein import mechanisms and probably causing dysfunction of complex I, although the data are not conclusive in this regard[46]. Dysfunction of

mitochondrial complex I has been shown in PD, and it is known to lead to cell death though the exact mechanism of this remains unknown[54]. Mitochondrial dysfunction may also occur with ageing, as deletions of mitochondrial DNA accumulate. Ageing may therefore increase the vulnerability of mitochondria to alpha-synuclein toxicity. Dysfunctional mitochondria produce ROS and further activate microglia. Also genetic studies point to the important role of mitochondrial dysfunction in PD. Some genes identified in the hereditary forms of PD, such as PINK1, LRRK2 and parkin, regulate the function of mitochondria and increase the vulnerability of mitochondria to ROS[55].

Increased load of alpha-synuclein also renders the dopaminergic cells vulnerable to exogenous toxins. The pesticides used to mimic PD in animal models of the disease are complex I inhibitors and their toxicity is aggravated by alpha-synuclein.

Although alpha-synuclein accumulation, aggregation and ensuing toxicity are important steps in the pathogenesis of PD, alpha-synuclein does not seem to be the sole culprit. The special features of dopamine metabolism suggest that its alterations also contribute to the process.

Dopamine degradation produces ROS, especially o–quinones, which are able to damage mitochondria, induce alpha-synuclein aggregation, alter protein degradation and increase oxidative stress[55]. Dopamine metabolism also requires iron as a cofactor[56]. While iron is essential to the normal functioning of all cells, an increased amount of unbound iron is highly toxic[57]. The iron content of the brain tends to increase during normal ageing, but this process is accelerated in PD, where iron accumulation has been found especially in the substantia nigra without a corresponding increase in the level of iron binding proteins such as ferritin[54] or, more importantly, neuromelanin[58].

The end-product of dopamine degradation is neuromelanin, an antioxidant and iron-binding protein[59,60], which is stored in dopaminergic neurons and gives substantia nigra its dark colour. When released into extracellular space after dopaminergic cell death, it induces an inflammatory reaction in the microglia. In living cells, neuromelanin helps to contain the oxidative burden produced by dopamine metabolism and is mainly responsible for sequestering free iron in the substantia nigra. In PD, the iron binding capacity of neuromelanin saturates and the pool of labile, unbound iron in nerve cells increases. The unbound iron contributes to the neurodegeneration via Fenton reaction, which produces cytotoxic hydroxyl radicals[57,61], increases the level of oxidative stress and promotes alpha-synuclein aggregation. Iron also interacts with the o-quinones to increase the pro-oxidant burden in the PD brain [56].

An epidemiological finding of special interest is that melanoma predicts an increased risk of PD[62]. The causal link has not been established, but melanin is

an obvious common denominator. A genetic defect or dysfunction of the melanin-related enzymes has been speculated to explain this association. Also phosphorylated alpha-synuclein has been found to be enriched in cutaneous malignant melanoma[63]. From these findings, it may be deduced that either there is an underlying malfunction of the melanins or alpha-synuclein changes the function of melanin in both melanoma and PD.

The pathogenic mechanisms described above form a vicious cycle which drives the neurodegeneration in PD. What initially induces the neurodegenerative process is still unclear, but research on the interplay of the intestine and brain may provide additional clues.

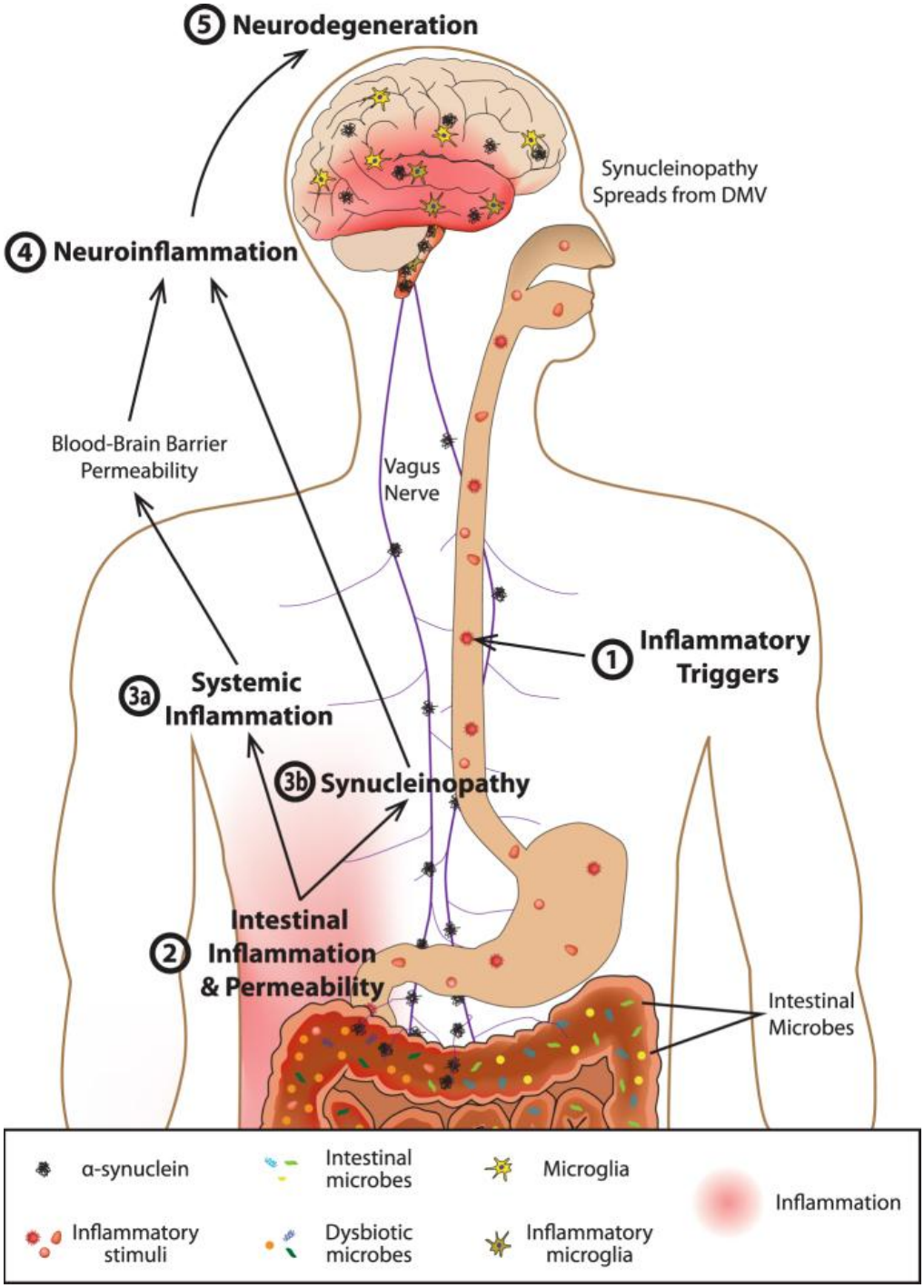
It has long been recognized that there is some form of gastrointestinal dysfunction in PD; chronic constipation is one of the strongest risk factors or more likely a prodromal symptom of the disease. Slow gastric emptying and decreased intestinal motility predispose PD patients to small intestine bacterial overgrowth (SIBO), the prevalence of which has been found to be significantly higher in PD patients than in normal controls (54% vs. 8%)[64]. In addition, antimicrobial therapy has been reported to improve the motor symptoms in the patients with SIBO[65]. Against this background, the novel hypothesis of gut-originating, inflammation-driven PD presented by Houser et al.[66] gives a new perspective to the PD puzzle.

Houser et al. propose that the first step of PD is an inflammatory trigger introduced in the gut. This may be a toxic substance, such as a pesticide, or an infectious agent that provokes an intestinal inflammatory response. In some, perhaps genetically susceptible individuals, this leads to chronic intestinal inflammation, increased permeability of the gut wall and deleterious changes in the microbial composition of the intestine.

Altered gut microbiota has been recently described in a Finnish study reporting that in the faeces of PD patients the abundance of Prevottellaceae was reduced, and, on the other hand, in those with postural instability and gait disturbance the abundance of Enterobacteriacea was increased[67]. The authors suggested that gut microbiota alteration may change the behaviour of enteric neurons and possibly increase the secretion of alpha-synuclein. The vagal nerve would be a possible pathway through which alpha-synuclein would then spread to the brain. In support of this theory, alpha-synuclein has been found in the parasympathetic intestinal plexuses of Meissner and Auerbach. Also a proof-of-concept study was recently carried out where human alpha-synuclein was introduced to the rat intestinal wall and later found in the brain stem[68].

In the classical Braak's model of PD neuropathology, DMV is the site of the first appearance of alpha-synuclein. However, Braak's model has been challenged by Kalaitzakis et al., who noted that not all PD brains show alpha-

synuclein pathology in DVM[69]. This contradicts the theory that the vagal nerve plays an essential role in transferring noxious substances to the brain in PD. If intestinal pathology has a role in PD, it seems to be more likely that the inflammatory mediators leak from the damaged gut wall to provoke a systemic immune response and damage the blood-brain barrier. This eases the entrance of the mediators of inflammation into the brain, where local microglia-driven inflammation induces alpha-synuclein aggregation. A study by Donadio et al. demonstrated phosphorylated alpha-synuclein in skin-nerve biopsies in patients with PD, thus favouring the systemic mechanism in the pathogenesis of PD[70].



Picture 1. Model of gut-originating, inflammation-driven PD pathogenesis. Reprinted from the original article with the permission of Dr. Tansey.

In summary, while multiple pathogenic mechanisms have been recognized in PD and there is increasing evidence of their interplay at least in the brain and probably also elsewhere in the body, the triggering event remains obscure. It may be that the mechanism that has evolved to protect the brain from the harmful by-products of dopamine metabolism fails due to genetic or environmental factors or ageing, or more likely due to a combination of these factors. There is also a possibility of an unrecognized exogenous trigger that might be a neurotropic virus, toxic substance or even a prion-like protein that provokes inflammation in the brain and possibly in the gut, increases the level of alpha-synuclein in the intestine and peripheral and central nervous systems, activates microglia and produces chronic inflammation in the brain. In individuals with genetic susceptibility, age-related accumulation of mutations in the mitochondrial genome and lifestyle factors predisposing to PD, the inflammation accelerates oxidative stress, mitochondrial dysfunction and accumulation of iron in the substantia nigra.

Uric acid in pathogenesis of PD

Low serum uric acid level is a risk factor for PD. A low level of uric acid may be explained by genetic or dietary factors, BMI and possibly altered microbial environment of the gut. Prevotella-dominated enterotype, rare in PD patients, is associated with higher uric acid levels. The reason for this in not known, but individuals with abundant Prevotella bacteria may have lower activity of hydroxyl-isourate hydrolase, which catalyses intestinal uricolysis[71]. Prevotellae also produce butyrate, a short-chain fatty acid that has anti-inflammatory actions and this may also affect colonic uric acid level[71]. Currently, it is not known whether the changed microbiota of the gut is a cause or a consequence of PD. It may also be an unrelated chance finding. However, it is an intriguing thought that changes in the gut microbiota would predispose to developing PD also by reducing the brain's antioxidant capacity by lowering uric acid levels. This would also mean that individuals with genetically low plasma uric acid levels would be more vulnerable to changes in the intestinal microenvironment.

In PD, one of the central pathogenic mechanisms is oxidative stress, which is partially a consequence of an increased pool of labile iron in the brain. The neuroprotective action of uric acid has been attributed to its antioxidant and iron binding capacity[72-74]. In dopaminergic cellular models, uric acid has prevented oxidative stress and cell death caused by 6-hydroxydopamine, dopamine and rotenone (a pesticide used to induce neuropathological changes similar to PD). Uric acid is able to form stable complexes with iron ions, thus reducing the toxicity of unbound iron[72]. Serum uric acid and ferritin concentrations are closely correlated[75], which indicates common regulatory mechanisms of their

production, and uric acid and ferritin have shared responsibilities in the human body and brain[75,76].

Uric acid also indirectly increases the antioxidant capacity of the brain by regulating astrocytes via nuclear factor like 2 (Nrf2) pathway[77]. Nrf2 transcriptionally regulates the expression of gamma-glutamyl transferase, the rate-limiting enzyme of glutathione synthesis. Glutathione is a powerful antioxidant, which is released by astrocytes as a reaction to oxidative stress. Astrocytes also express glutathione transferase, which catalyses glutathione conjugation of the o-quinones into stable forms. This mechanism probably protects both astrocytes and dopaminergic neurons from the oxidative by-products of dopamine metabolism[78].

In summary, uric acid seems to be a strong modifier of the risk of PD. Low serum uric acid level, the cause of which may be genetic, dietary or possibly related to altered gut microbiota, increases the risk of PD. A low level of serum uric acid renders the brain more vulnerable to increased oxidative stress caused by dopamine metabolism, alpha-synuclein aggregation, mitochondrial dysfunction and neuroinflammation. The neuroprotection provided by uric acid is probably mediated by the Nrf2 pathway, but uric acid's direct antioxidant function and iron binding capacity may also be important features.

Recent clinical findings of uric acid in PD

The PRECEPT study recruited patients with early PD to investigate the effect of an orally administered anti-apoptotic mixed lineage kinase inhibitor CEP-1347, which was assumed to be neuroprotective based on animal data. The results were not promising, but serum uric acid concentration was measured at baseline in 806 PD patients and in the sub-analyses it appeared to predict clinical outcome. The study subjects were followed up for an average of 21.4 months, during which time 61% reached the primary end point of disability sufficient to require dopaminergic therapy. The higher the uric acid level at baseline, the less likely it was to reach this end point. The association was stronger in men than in women.

Single-photon emission tomography of iodine I 123-labelled beta-CIT uptake was available in 399 patients at both baseline and end of follow-up. The progression of dopaminergic deficit was also slower with high serum uric acid levels, and, again, the association was stronger in men. The authors concluded that serum uric acid is the first molecular factor directly linked to the progression of typical sporadic PD[79].

Later, the same researchers repeated their findings in another study that was designed to determine whether serum and cerebrospinal fluid uric acid levels would affect clinical progression of PD. The subjects were again people with early PD and the primary end point was disability requiring levodopa therapy. Higher serum and cerebrospinal fluid uric acid concentrations were associated with slower rates of clinical decline[79,80].

In their third study on the subject, the authors found that in patients with parkinsonism who were included in the study as PD patients, the likelihood of having a (123I)beta-cit SPECT without dopaminergic defect was higher those with high serum uric acid concentrations[81].

Increasing uric acid as a therapeutic strategy in PD

The promising data of the above-mentioned study inspired a trial to use inosine to increase the serum uric acid levels in PD[39]. This phase II trial aimed to assess safety and tolerability of serum uric acid elevation in early PD not yet requiring symptomatic therapy.

Seventy-five patients with below population median serum uric acid levels were recruited and randomized to either placebo or a low or high dose of inosine. The patients with above population median serum uric acid levels were excluded because the net benefit concerning the risk of progression might not be significant in that patient group and also because they were considered more

vulnerable to the possible adverse events caused by increased serum uric acid concentration (gout, urolithiasis, renal disease and vascular events).

In the active treatment group, there was an increased number of urolithiasis cases, but no serious adverse events associated with high serum uric acid concentration occurred; blood pressure, BMI, serum glucose and cholesterol levels remained the same throughout the study. In the study group, the serum uric acid levels were significantly increased relative to placebo. Importantly,, there was a trend towards slower progression of the disease in the active treatment group. The study population was small and the follow-up short, bearing in mind the slow progression of PD. However, the results concerning safety and tolerability were promising. A phase 3 trial (NCT02642393) is now ongoing to assess whether increasing serum uric acid levels with inosine might decelerate the progression of motor symptoms in early PD. This randomized, placebo-controlled, double-blind, multicentre trial is planned to last 2 years and the main outcome variable is change in the Movement Disorder Society's Unified Parkinson's Disease Rating Scale (MDS-UPDRS).

Cognitive decline in PD

Cognitive decline is one of the most devastating symptoms of PD. The studies examining cognition in early, non-medicated PD are few but well conducted. The estimations of the prevalence of mild cognitive impairment (PD-MCI) vary in these studies, ranging between 18% and 34% [82] [83] [84]. The variance is probably due to differences in patient demographics and study designs. Moreover, the diagnostic criteria for PD-MCI have not been consistent, but this problem will probably be mitigated in future studies that will hopefully follow the Movement Disorder Society Task Force Guidelines to diagnose PD-MCI[85].

The most common neuropsychological deficits in PD are found in the areas of attention, verbal memory and fluency, psychomotor speed and visuospatial and executive functions[83,86-88]. Once established, PD-MCI carries a poor prognosis with further decline in cognition and subsequent development of PD-Dementia (PDD)[6]. There are two quite recent studies on the evolution of cognitive decline in early PD that follow the Movement Disorder Society Task Force Guidelines to diagnose PD-MCI and PDD[89]. Both of these have extensive follow-ups.

In the Norwegian Park-West Study, 182 patients were serially tested for neuropsychological deficits at baseline and after 1 and 3 years. Of the patients, 20.3% were considered to have PD-MCI at baseline, 27% of whom developed PDD within 3 years of follow-up. The demented patients were older, less educated and had longer disease duration and more severe motor symptoms of PD than the non-demented group [90].

In another study, 123 PD patients were examined at baseline. Due to drop-outs, 93 and 59 patients were available for follow-up at 3 and 5 years, respectively. Altogether, 35% were found to have PD-MCI at baseline and 53% at 3 years. Of the 59 patients who reached the final follow-up visit, 50% had PD-MCI and 34% PDD[4].

The neuropathology of cognitive decline in PD includes degeneration of subcortical nuclei with both neuronal loss and Lewy body pathology. The magnitude of cell loss in the medial substantia nigra and ensuing dopaminergic defect correlate with the severity of cognitive decline. Also locus coeruleus, nucleus basalis of Meynert and dorsal raphe nucleus degenerate in most cases. These nuclei have vast connections to cortical areas and their degeneration leads to cortical noradrenergic, cholinergic and serotonergic defect, respectively[91]. The ensuing corticostriatal dysfunction explains the clinical picture of diminished attention, dysexecution, visuospatial problems and reduced working memory capacity that are common in PD.

Cortical Lewy body pathology as suggested by Braak is not always present even in PDD, and, on the other hand, patients even in Braak stages 5-6 may not have clinically defined dementia[92]. The major risk factor for cognitive decline and dementia in PD is age[93]. While cognitive decline is not a part of normal ageing, pathologies other than PD may accumulate over time. Concomitant Alzheimer's disease (AD) and cerebral vascular pathology lower the threshold for the appearance of cognitive symptoms in PD. Genetic findings also corroborate the hypothesis of multiple pathogenic mechanisms of cognitive decline since PD patients with polymorphisms in cathecol-O-methyl-transferase (COMT), microtubule-associated protein Tau (MAPT) and APOe have a predilection for distinctive cognitive profiles[94]. COMT regulates the level of cortical dopamine, MAPT regulates the folding of Tau-protein and APoe4 predisposes to AD dementia.

One must also bear in mind that there are protective factors, such as education and physical activity, which may help to preserve cognition even in neuropathologically advanced disease.

There is no reliable biomarker for the diagnosis of PD-MCI or PDD. In neuroimaging studies, widespread hypometabolism has been described in frontal, temporal, parietal and occipital cortical regions [95,96]. In PDD, these changes usually exceed the metabolic changes found in AD, reflecting a global neurotransmitter deficit. Magnetic resonance imaging (MRI) studies have found cortical atrophy in the same regions as well as hippocampal atrophy, although to a lesser extent than in AD [9798].

PET imaging with Pittsburgh compound to detect cortical amyloid has also been examined in PD. Interestingly the amyloid load is significantly lighter in PDD

than in AD, and in cognitively normal PD it seems to be similar to that in healthy controls [99]. However, it has been hypothesized that even a low amyloid burden would have a marked impact on cognition with coexisting PD. The underlying neurodegenerative process makes the brain more vulnerable to amyloid and ensuing memory problems. Interestingly, although neuropathology in dementia with Lewy bodies is difficult to differentiate from PDD, there is a higher prevalence of AD pathology in dementia with Lewy bodies[100]. Clinically, dementia with Lewy bodies and PDD bear a close resemblance and their differential diagnosis is rather arbitrarily defined by the sequence of the occurrence of symptoms. If the cognitive symptoms precede the onset of the motor symptoms by a year or more, the disease is called dementia with Lewy bodies[101]. If the motor symptoms appear first with ensuing cognitive decline, the disease is called PDD[89]. These two diagnoses have lately been considered to represent the opposite ends of a disease spectrum and an umbrella term of Lewy body dementias has been suggested to include both dementia with Lewy bodies and PDD[102]. In my opinion, dementia with Lewy bodies may be seen as a very late onset form of PD with coexisting AD pathology.

Uric acid and cognition

While the neuroprotection offered by uric acid against developing PD and progression of motor symptoms seems undisputable, the evidence concerning cognition is contradictory.

As to the available literature, the first study assessing uric acid's effect on cognition was conducted in 2007 by Schretlen et al.[103] In this community-based study, 96 adults over 60 years of age and with no evidence of neurological disease such as PD were examined. All of the study subjects had serum uric acid levels within the reference range, but those with high-normal levels scored poorly in tasks measuring verbal and working memory. This association remained statistically significant even after controlling for several potential confounders such as age, sex, education, diabetes, hypertension, smoking and abuse of alcohol. The authors suggested that the detrimental effect of uric acid on cognition might be mediated by cerebrovascular changes, as high serum uric acid has been considered a vascular risk factor associated with diabetes, hypertension and obesity.

Schretlen et al. tested their hypothesis in another community-based sample of 177 subjects aged 20-92 years. The amount of cortical and subcortical hyperintense white matter lesions was evaluated from brain MRI scans and these lesions were considered to be of vascular origin since patients with other causes of white matter lesions, such as multiple sclerosis, were excluded. The serum uric acid levels were within the reference range, but even a high-normal uric acid level increased the likelihood of a heavy ischaemic burden up to 5-fold in subjects aged 60 years and over.[104] This finding was corroborated in the Rotterdam Scan study, where white matter atrophy and worse cognition were associated with hyperuricaemia in 814 people from a community sample[105].

Euser et al. conducted a large prospective community-based study including 4618 subjects aged 55 years or older to investigate the relationship between uric acid and subsequent dementia. During the 10-year follow-up 457 subjects developed dementia. Ten years after the baseline serum uric acid measurement, the cognitive function was analysed in a subpopulation of 1724 subjects who remained free of dementia. After carefully controlling for several vascular risk factors, higher uric acid levels at baseline were found to be associated with later decreased risk of dementia and better cognitive function.[106]

The conclusion from these population-based studies is that uric acid is probably beneficial with respect to cognition at least in individuals without substantial vascular risk factors. It is not known whether uric acid is only a secondary marker of vascular disease in people with several vascular risk factors, such as obesity, diabetes, hypertension and hypercholesterolaemia, as uric acid levels are known to be increased in these states. An alternative explanation is that the

function of uric acid is altered in the vascular endothelium during atherosclerosis. At the beginning of this process, uric acid has a major contribution to the plasma antioxidant capacity. As atherosclerosis advances, the pH of vascular endothelium and intima becomes acidic, local antioxidants are depleted and ROS cause an ongoing oxidative stress. In this milieu, the antioxidant properties of uric acid may change into pro-oxidant in a reaction called antioxidant/pro-oxidant redox shuttle [107]. This theory would be in conjunction with epidemiological findings presented above.

In addition to our studies concerning uric acid and cognition in PD, there is only one study exploring this subject. Moccia et al. recruited 80 patients with newly diagnosed PD. The study subjects filled in a validated questionnaire (NMSQuest) to detect non-motor symptoms of PD. The authors reported that higher serum uric acid levels were associated with better attention and memory at the early stages of the disease [108]. At the follow-up evaluation, this association remained strong; the patients with high serum uric acid levels reported fewer problems in the attention and memory domain of NMSQuest [109]. The obvious limitation of this study was the lack of a neuropsychological examination.

While the findings of Moccia et al. seem promising, the role of uric acid in neurocognitive changes in PD remains unresolved.

Event-related potential findings in PD

Event-related potentials (ERPs) during electroencephalogram (EEG) recording are used to detect visual or auditory information processing in the brain[110]. The focus of this review is on the auditory modality.

An ERP is a cortical response time locked to a sensory stimulus or a mental occurrence presented during EEG recording. The stimuli can be expected by the patient, who is advised to focus on certain kinds of sounds (target) and to ignore others (standard or non-target). An unexpected and often strange sound is called a novel stimulus or novelty. ERP contains positive and negative waves that are named according to their latency and polarity. N100 is a negative response that occurs approximately 100 ms after the stimulus, and P200 is a positive response that occurs approximately 200 ms after the stimulus. ERPs can be used to examine attention, memory and language[111].

A non-target sound usually elicits N100 and P200 responses, indicating that the stimulus has been noted. The N100 describes activity of both auditory and frontal cortices[112] and correlates with attention and also with filtering out irrelevant information; once noted, a standard tone does not require further

cognitive processing and can be forgotten[113]. The magnitude of the N100 response also declines upon repetition of the stimulus, a phenomenon called habituation[114].

A target sound elicits a N200-P300b complex, reflecting conscious stimulus processing in the brain. A novel surprise sound causes a larger and earlier P300a wave that is considered to describe an orienting response to a new situation and ensuing cognitive processing[111].

The P300 component of EEG-ERP is considered most sensitive to detect cognitive impairment in various patient populations, and it is also the most extensively studied in PD. The latency of the P300 response has been reported to be prolonged in PD,[115] and especially in PD-MCI and PDD, where the response is often absent altogether. These findings are very consistent, but similar findings have been described in amnestic MCI and AD, thus reducing the specificity of P300 in detecting PD-related cognitive impairment.

N100 and P200 potentials have gained less research interest in PD, and the findings have been inconsistent. Both the N100 and P200 latencies have been measured as normal[118-120] or prolonged[114,121-123] in PD. The only previous study comparing the N100 potential and neuropsychological examination was conducted by Raudino et al.,[123] who failed to show an association.

An explanation for these inconsistencies may be methodological variation and a limited number of study subjects. Also the patient populations described have been quite heterogeneous concerning age as well as severity and duration of the disease.

What makes the N100 potential an intriguing research subject in PD is its role in describing attentive processes[87,113,124]. Attention is not just a simple reaction, but a complex, partly subconscious process that has developed to enable tracking of relevant sensory information and ignoring irrelevancies. Being able to sustain attention also contributes to working memory by preventing redundant stimuli being processed and stored even temporarily. Attention is under the control of the basal ganglia and often impaired early in the course of PD[125]. Attentional impairment may therefore also partly explain the poor working memory often noted in early PD[126,127]

Although EEG-ERP measurements would provide objective information on the stimulus processing in the brain, there are no clinical applications to aid in the diagnosis of neurodegenerative diseases. The inconsistent results from previous studies and rather low specificity for PD have probably hindered willingness to develop this method in clinical practice. However, there are certain practical aspects of EEG-ERP that might make it beneficial in PD populations. EEG-ERP does not require any physical effort from the patient; PD patients usually suffer from slowness of movement and poor manual dexterity. EEG-ERPs are

non-invasive, relatively easy to measure and inexpensive compared with a neuropsychological examination, which patients often consider exhaustive. Test-retest reliability is good,[128] and especially the N100 potential and to some extent also P200 seem to be independent of age[128,129]. The role of N100 as a sensory gatekeeper links it to the neuropsychological defects that are common in PD. Essential for future studies would be to select more homogeneous patient populations concerning especially the duration and severity of the disease.

Aims of the study

This thesis consists of four studies, which are later referred to as Studies I, II, III and IV.

Study I was conducted to examine whether uric acid, considered both an antioxidant and an iron binding molecule and having neurocognitive properties in experimental studies, is diminished in the plasma or urine of PD patients compared with normal controls.

Study II aimed to determine whether an association exists between plasma/urine uric acid level and cognitive changes in non-demented PD patients.

Study III aimed to examine whether a low level of plasma/urine uric acid would predict faster cognitive decline in PD after 3 years of follow-up.

Study IV aimed to examine the usefulness of neurophysiological measurements in evaluating cognition in PD and whether uric acid levels are associated with the neurophysiological parameters measured.

List of original publications

I Low plasma uric acid level in Parkinson's disease.
Annanmaki T, Muuronen A, Murros K.
Mov. Disord. 2007 Jun 15;22(8):1133-7.

II Uric acid associates with cognition in Parkinson's disease.
Annanmaki T, Pessala-driver A, Hokkanen L, Murros K.
Parkinsonism Relat. Disord. 2008 Nov;14(7):576-8.

III Uric acid and cognition in Parkinson's disease: a follow-up study.
Annanmaki T, Pohja M, Parviainen T, Hakkinen P, Murros K.
Parkinsonism Relat. Disord. 2011 Jun;17(5):333-7.

IV Altered N100-potential associates with working memory impairment in Parkinson's disease.
Annanmaki T, Palmu K, Murros K, Partanen J.
J Neural Transm 2017 Oct;124(10):1197-1203.

Description of Studies I-IV

Studies I-IV are prospective studies including PD patients from the neurological outpatient clinic of Jorvi Hospital.

Inclusion criteria for Studies I-III were idiopathic PD as defined in the UK Parkinson's disease Brain Bank clinical diagnostic criteria, age 50-70 years and disease duration from diagnosis of no more than 10 years. The control group was formed by patients' spouses, who were age-matched and had no history of PD. Exclusion criteria for both the patients and controls were current use of medications affecting plasma uric levels, current iron metabolic disorder, dementia and refusal to participate.

For Study IV, the inclusion criteria were idiopathic PD as defined in the UK Parkinson's disease Brain Bank clinical diagnostic criteria and no clinical signs of dementia. The exclusion criterion for patients was current use of a medication other than levodopa that affects plasma uric acid levels. The controls were healthy volunteers for whom the inclusion criterion was a normal result in the Consortium to establish Alzheimer's disease (Cerad) test series to exclude mild cognitive impairment and dementia, and the exclusion criterion was any clinical sign of neurological disease.

Studies I-IV were approved by the Ethics Committee of the Health District of Helsinki and Uusimaa. All patients and controls signed a written informed consent form.

Study I

Materials and Methods

Study I was planned to include 40 PD patients and their eligible spouses, whom we were able to recruit during a 9-month period starting in February 2005. In the patient group, the mean disease duration from diagnosis was 4.1 years and the mean motor UPDRS score was 18.8 points (range 5-35). All patients were on medication; 28 on levodopa with a median daily dose of 415 mg, 28 on dopamine agonist and 31 on selegiline. Levodopa equivalent daily dose values were not calculated.

Fasting blood samples were collected from both patients and controls to assess the levels of uric acid, ferritin, transferrin, transferrin saturation, transferrin receptor, iron, haemoglobin, ceruloplasmin and creatinine. All urine excreted over 24 hours was collected to analyse urine uric acid concentration.

As dietary factors are known to affect plasma uric acid levels, a dietary recall diary was kept for 4 days to control for this confounder. Also weight affects plasma uric acid levels, and therefore, weight was measured and the patients and controls reported their height to calculate body mass index (BMI). The demographic characteristics and laboratory findings are presented in Table 1.

Table 1.

Demographic characteristics and laboratory findings of patients and controls.

	Study group n=40	Control group n=29	Reference range
Mean age, years (SD)	60.8 (6.5)	60.2 (5.1)	
Sex, men (%)	58	45	
MMSE score, mean (range)	28 (25-30)	28 (23-30)	
Hypertension, prevalence (%)	28%	17%	
Smoking, prevalence (%)	8%	0%	
Plasma uric acid, umol/l	278.5 (77.4)*	319.7 (76.0)*	155-400 (women) 230-480 (men)
Urine uric acid, umol/l	3.2 (1.1)**	3.5 (1.1)	3-6
Creatinine, umol/l, mean (SD)	71 (12.4)	70 (11.3)	58-100
Haemoglobin, g/l	141.4 (11.6)	144.8 (9.4)	117-167
Ferritin, ug/l	84.6 (62.3)	91.1 (83.0)	5-200
Transferrin, g/l	2.6 (0.4)	2.5 (0.3)	1.75-3.13
Transferrin saturation, %	27.8 (9.4)	32.0 (10.2)	17-52
Transferrin receptor, mg/l	1.2 (0.5)	1.1 (0.3)	0.9-2.3
Iron, umol/l	18.2 (5.5)	20.5 (6.3)	9-34
Ceruloplasmin, mg/l	310.3 (46.8)	301.1 (68.8)	200-550

Means, standard deviation in parentheses.

* T-test: p=0.03, inter-assay variation 2.8-3.4%

** n=37

Results

The main finding of Study I was that PD patients had significantly lower levels of plasma uric acid levels than controls. The finding was not explained by differences in dietary patterns or BMI. Based on the dietary recall diaries, the patients and controls consumed similar amounts of calories, fats, carbohydrates, proteins, cholesterol, vitamins A, B12, C, D and E; also the amounts of iron, zinc and selenium were similar.

BMI was lower in patients than in controls, and this finding was of borderline significance (T-test, P=0.05). Expectedly, BMI correlated strongly with plasma uric levels in both the patient and control groups. To control for BMI and other factors possibly confounding the results regarding uric acid levels, a stepwise multiple linear regression was used. Age, gender, BMI, ferritin and PD were all found to contribute significantly to plasma uric acid level ($p=0.0001$ for the model).

No differences emerged between patients and controls in the markers of peripheral iron metabolism. Plasma uric acid level showed a strong correlation with serum ferritin level ($r=0.52$, $p=0.001$).

Study II

Materials and Methods

In Study II, the same patient population as in Study I underwent a thorough neuropsychological examination. The tests were chosen from the Wechsler Adult Intelligence Scale, Revised, Wechsler Memory Scale, Third Edition and Behavioral Assessment of the Dysexecutive syndrome to assess visual and verbal working memory, visuospatial and visuoconstructive abilities, verbal fluency and executive functions. Mood was evaluated with Beck Depression Inventory (BDI).

In addition to the traditional neuropsychological tests, computerized tasks with Cognispeed software were used. Cognispeed was developed in Finland to be used in both linical and research settings to examine certain aspects of cognition. The testing situation is assisted by a psychologist familiar with the software. One of the central aims of Cognispeed is to differentiate between automatic and controlled information processing. The tasks are planned to measure speed and span of working memory and the time to make the appropriate decision in multiple choice situations. It also examines different aspects of attention such as dividing attention between simultaneous memory and decision tasks, concentrating attention in the subtraction task, suppressing redundant information in the Stroop type task and being able to sustain and focus attention on a certain stimulus and reject others in a vigilance task lasting 15 minutes. Considering the attentional and working memory problems often

detected in PD, Cognispeed was considered a valuable addition to the traditional neuropsychological examination in our patient sample.

Results

The main finding of Study II was the correlations of both urine and plasma uric acid levels with several cognitive measures as shown in Table 2.

Table 2.

Correlations of urine- and plasma uric acid with neuropsychological tests

Test	Pearson Correlation with urine uric acid	Pearson Correlation with plasma uric acid
General cognitive status		
MMSE		
Information	0.37	
Similarities	0.40	0.36
Visual functions		
Block design	0.41	
Picture completion	0.46	0.37
Learning and memory		
Digit span forward		
Digit span backward	0.33	
Digit symbol		
Logical memory		
Word list		
Visual reproduction		
Executive functions		
Trail making A		
Rule shift cards	0.34	0.09
Verbal fluency		
Animals		0.10
Words with K		-0.21
Cognispeed		
SRT		
2-CRT		
10-CRT	-0.32	
Subtraction		
Statement verification	-0.58	-0.38
Cognitive processing	-0.58	-0.37
Stroop type task		
Vigilance (n=34)	-0.44	-0.41

Only the significant correlations with $p<0.05$ are shown

MMSE=Mini mental state examination

SRT= Simple reaction time

2-CRT= 2-choice reaction time; 10-CRT=10-choice reaction time

To control for possible confounding factors that might also contribute to cognitive performance, a multiple linear regression was used. The possible confounders taken into account in the analysis were age, gender, disease duration from diagnosis, plasma homocysteine, depression, education, levodopa therapy and BMI.

In the multiple regression analysis, urine uric acid was the only variable that predicted both reaction time and time spent on cognitive processing in the Statement verification task
($F = 16.5$, $R^2 = 0.31$, $p = 0.0001$; $F = 17.3$, $R^2 = 0.33$, $p = 0.0001$, respectively) as well as achievement in the Picture Completion test
($F = 10.1$, $R^2 = 0.21$, $p = 0.003$). Urine uric acid also had a small but significant contribution to the Rule shift cards and Block Design scores. Education, gender and depression accounted for some of the variation observed in the neuropsychological parameters.

Also plasma uric acid was found to be associated with the Statement verification task together with education ($F = 6.2$, $R^2 = 0.26$, $p = 0.005$ for reaction time and $F = 7.6$, $R^2 = 0.3$, $p = 0.002$ for time required for cognitive processing during the task). Plasma uric acid and education contributed to the Similarities subtest ($F = 5.7$, $R^2 = 0.24$, $p = 0.007$).

In summary, both urine and plasma uric acid seemed to have small but significant effects on the speed of cognitive processing as well as on achievement in the tasks requiring visuoconstructive abilities and visual memory.

Study III

Material and Methods

After three years of follow-up, the same research protocol was repeated in Study III. Neuropsychological evaluation was performed on patients and both patients and controls underwent laboratory testing and completed a 4-day dietary recall diary. As a novel feature, the computerized tasks with Cognispeed were performed on both patients and controls. Unfortunately, several patients were lost to follow-up for reasons other than PD, and only 28 patients and 13 spouse controls were examined.

Results

There was no significant change in plasma or urine uric acid levels during follow-up. BMI and eating habits also remained stable. Surprisingly, there were only minor cognitive changes in the patient group. A significant deterioration was noted in Verbal fluency (p=0.04) and Cognispeed's vigilance task (p=0.0001). No significant change occurred in Cognispeed's statement verification task, but the time required for cognitive processing during the task was lengthened ($p = 0.03$).

When patient data on Cognispeed were compared with control data, a significant difference was present in Stroop-type colour-word reading task (p=0.05), delayed visual reproduction task of WMS-R (p=0.01) and both simple and two-choice reaction times ($p = 0.03$ and $p = 0.004$, respectively).

After controlling for possible confounding factors, such as age, gender, education, disease duration from diagnosis and BDI, with forward linear regression, only the baseline urine uric acid seemed to contribute to neuropsychological performance. It was associated with verbal fluency ($R^2 = 0.15$, $F = 4.6$, $p = 0.04$), picture completion ($R^2 = 0.35$, $F = 13.9$, $p = 0.001$), block design ($R^2 = 0.28$, $F = 10.0$, $p = 0.004$), vigilance ($R^2 = 0.27$, $F = 9.7$, $p = 0.004$), subtraction ($R^2 = 0.23$, $F = 7.5$, $p = 0.01$) and statement verification ($R^2 = 0.28$, $F = 10.0$, $p = 0.004$) tasks. The correlations of the baseline plasma and follow-up plasma and urine uric acid levels with cognition seemed non-significant.

Study IV

Materials and Methods

For Study IV, we recruited 14 patients from the above-described patient population and, due to dropouts in the primary sample, 4 additional patients from the neurological outpatient clinic of Jorvi Hospital. The controls were members of the hospital staff and their acquaintances.

For this final study, 18 patients and 24 controls underwent EEG-ERP measurement. Recording and quantification of EEG and stimuli to induce ERP were done with a Cognitrace EEG/ERP system, version 3.3.

During the ERP recording the patients were asked to follow a silent nature movie shown on a TV screen at a distance of 1.5 metres. Auditory stimuli were given through calibrated headphones (Telephonics TDH-39P), and the patients were instructed not to pay attention to the tones. The auditory ERPs were stimulated with pairs of tones with an inter-stimulus interval (ISI) of 0.5 and 3 seconds, as well as novelty sounds. The responses to first and second tones of pairs with 0.5 second ISI were analysed. ERP responses for 40 pairs of stimuli were averaged.

The amplitude and latency of N100 (Cz) and P200 (Cz) elicited by the auditory stimuli were measured with respect to the highest negativity in a 60-170 ms post-stimulus time interval and the highest positivity in a 100-260 ms interval. Habituation of the N100 was calculated as the difference between the amplitudes elicited by the first and second tones of a pair with 0.5 second ISI.

Neuropsychological data from the previous study were available for 15 patients and the plasma uric acid measurement for 16 patients. The Consortium to establish Alzheimer's disease (Cerad) test series was conducted for controls to rule out cognitive decline; a normal result was required for the subject to be included in the control group.

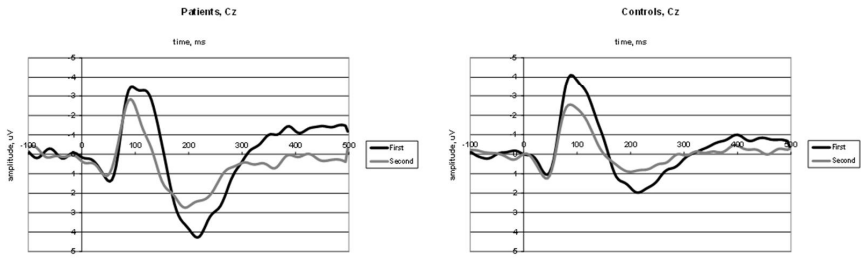
Results

Demographic characteristics of patients and controls as well as latencies and amplitudes of the N100 and P200 potentials are presented in Table 3.

Table 3.

	PD patients (n=18)	Controls (n=24)
Age (range)	62 (42-71)	67 (60-79)
Gender , % of males	44%	33%
MMSE (range)	28 (23-30)	28 (28-30)
Duration of disease in years, mean (range)	6 (2-13)	
UPDRS-motor, mean (range)	17 (1-51)	
Levodopa usage	13	
Levodopa dose, mean (range)	425 mg (250-875mg)	
Dopamine agonist	12	
Selegiline	13	
N100 Latency, mean (SD)	107.6 ms (23.2)*	95.1 ms (16.6)*
N100 Amplitude, mean (SD)	-5.5 uV (2.0)	-5.3 uV (2.0)
P200 Latency, mean (SD)	208.5 ms (30.7)	214.6 ms (21.5)
P200 Amplitude, mean (SD)	5.3 uV (2.8)	4.3 uV (1.7)

The N100 latency was significantly prolonged in patients relative to controls (Mann-Whitney test, p=0.005). With visual assessment, the change in the N100 amplitude between responses to the first and second tones of a pair of tones, called habituation, was more pronounced in controls than in patients, although this difference did not reach statistical significance. The grand averages of patients and controls are shown in the picture.



Prolongation of the N100 latency and reduced habituation of the N100 amplitude were associated with the delayed visual reproduction task of the WMS-R (Spearman rank -0.62, p=0.001). The patients were further divided into two groups according to the median score of this neuropsychological test result. The patients with an above median result had shorter N100 latencies (Mann-Whitney p=0.007) and stronger habituation of the N100 amplitude (Mann-Whitney p=0.004).

No correlation existed between N100 latency or amplitude habituation and plasma uric acid level in the patients.

Discussion of Studies I-IV

In Study I, we were able to show that PD patients had lower levels of plasma uric acid than normal controls. This finding remained significant after carefully controlling for several possible confounding factors. Of the confounders, BMI was lower in patients than in controls, and this finding was of borderline significance (p=0.05). Plasma uric acid level correlates with BMI, and it cannot be excluded that this difference in BMI might have had an effect on the results. In that case, low uric acid level might only be a secondary marker of the weight loss commonly seen in PD, especially in the more advanced stages. Considering that the patients and controls had similar dietary patterns and energy intakes, the lower BMI in the patients may also reflect gastrointestinal dysfunction and malabsorption of nutrients. Previous studies have shown that PD patients' energy intake is high and expenditure low, indicating that the patients are incapable of exploiting all energy consumed. It is also possible that some important nutrients, such as purines needed to produce uric acid, are malabsorbed in PD, or that the altered microbiome and intestinal inflammation accelerate the degradation of uric acid in the intestine, as suggested by Scheperjans et al. In that case, the disease-related pathology in the intestine would at least partially explain the low plasma uric acid levels in the patients.

Two studies have confirmed our results concerning plasma uric acid levels in PD[130,131]. They have both found a strong correlation between plasma uric acid levels and disease duration, which we did not take into account in the statistical analysis. This finding also points towards low plasma uric acid level being partially a consequence of the disease or perhaps the medication (levodopa), as suggested by the authors. Levodopa increases the secretion of uric acid by the kidneys. Considering the expanding evidence for a neuroprotective role of uric acid in PD, the possibility that the disease itself or symptomatic medication, e.g. levodopa, lowers plasma uric acid levels makes such therapeutic strategies as using inosine to increase plasma uric acid levels even more justified.

In Study I, we found no difference in markers of peripheral iron metabolism between patients and controls. According to the current knowledge, disorders of peripheral iron metabolism are not related to PD. This does not exclude the possibility of disturbed iron storage and transfer in the brain. In PD, there is an increase in the amount of iron in the substantia nigra and malfunction of local iron storage proteins such as neuromelanin. An excessive pool of unbound, labile iron probably accelerates the neurodegenerative process by increasing oxidative stress.

Our study could not resolve whether uric acid also has an impact on cognitive decline in PD. In Study II, we found both plasma and urine uric acid to be associated with cognition in PD. Interestingly, the associations concerned the areas of cognition that are often disturbed in PD, namely verbal memory and

speed of processing, visuospatial and visuoconstructive skills and executive function. The findings were so promising that they encouraged us to follow the study sample for three more years, resulting in Study III. Surprisingly, at the end of the follow-up, there was no significant deterioration in the cognitive profiles of the patients. Of the neuropsychological parameters, only verbal fluency, vigilance and speed of cognitive processing were significantly impaired relative to baseline evaluation. The computerized tasks were performed by both patients and controls; the only differences between the two groups were in Stroop-type colour-word reading task, delayed visual memory and reaction times.

In summary, the patients had subtle cognitive alteration typical for PD and some progression over the three-year follow-up. However, with respect to disease duration, they were indeed cognitively very well preserved. Cognitive defects or their progression did not seem strongly related to either baseline or current plasma or urine uric acid levels. The relative stability of neurocognitive symptoms in patients and the small sample size were also major obstacles in determining uric acid's possible protective effect on cognition.

The results of Study III may be partly explained by a chance selection bias; the patient population was largely formed by individuals with high socioeconomic status and educational level and with lifestyle factors contributing to the preservation of cognition [132]. Their cognitive reserve probably protected them from the somewhat faster cognitive decline usually observed in PD. Although we aimed to recruit consecutive patients, it is possible that the ones with more severe cognitive or neuropsychiatric symptoms declined to participate. Our patient sample may therefore not be fully representative of all PD patients.

The patients also visited a neurologist regularly and were advised to remain physically active, to avoid weight loss and to consume a protein-rich diet despite the limitations caused by levodopa medication. These actions decrease the likelihood of both cognitive decline and reduction in plasma uric acid levels.

Gender probably also had an effect. At the time of Study III, it was not yet established that the neuroprotective effect of uric acid is more pronounced in men than in women, in whom it may actually be non-existent[133,134]. To show uric acid's association with cognition in PD would therefore require a larger number of study subjects to be adequately powered or alternatively a study recruiting only male patients. Unfortunately, we also lost several patients to follow-up, which may have affected the results in such a small patient sample.

Uric acid's possible effect on cognition may also have been confounded by the variation in disease duration in our patient sample. In future studies, it would be important to recruit patients at similar disease stages and preferably already at the time of diagnosis.

The findings in healthy populations regarding uric acid's detrimental effect on cognition also warrant consideration. High serum uric acid level increases the risk of cerebral vascular lesions, and this association seems independent of other vascular risk factors such as hypertension, diabetes, smoking and obesity. Also in PD there is increasing evidence of vascular risk factors and cerebral vascular pathology contributing to cognitive decline and increasing the risk of PDD [135,136]. If uric acid gains pro-oxidant properties in atherosclerosis, its effect on cognition may be detrimental in PD patients with considerable cerebrovascular burden and neuroprotective in the ones with more classic PD-related brain pathology. Discerning these PD patient subpopulations in future studies will be a laborious undertaking, but probably necessary to disclose uric acid's true role in the cognitive decline of PD. Underlying vascular pathology may also be an unrecognized confounder in our study since we did not perform neuroimaging on our patients. However, vascular risk factors were rather uncommon; of the original 40 patients, 8 had a history of hypertension, 3 were current smokers and 1 had diabetes.

In Study IV, we found an interesting association of visual working memory with N100 latency prolongation and diminished amplitude habituation. This finding opens a window into the PD brain, shedding light on the interplay between attention, visual function and working memory or more accurately on the network between basal ganglia and frontal and occipital cortices. The heterogeneous mechanisms of cognitive decline and the large individual variation in both brain pathology and clinical picture of PD-MCI and PDD hinder the development of EEG-ERP for clinical use. However, the same problem concerns other biomarkers, such as MRI, PET and SPECT, the findings of which are not always in line with the individual clinical picture. Obvious limitations of Study IV were the small sample size and the large variation in disease duration.

Study IV was designed as a pilot and in that respect the results were promising considering the association of N100 potential with visual working memory. To develop a diagnostic tool to assess cognitive decline in PD would again require a larger number of patients in a similar disease stage. A clinical application might facilitate identification of PD patients at risk of cognitive decline even in the absence of cognitive symptoms or neuroimaging findings of cerebral vascular pathology. Identifying these patients might help in guiding treatment decisions such as initiating inosine to increase plasma uric levels.

In Study IV, we found no correlation between measured EEG-ERP parameters and uric acid. In light of the results of Study III, this was an expected finding. To show an association with a neurophysiological parameter measuring a narrow area of cognition would require a very strong association of uric acid with cognition, and this does not seem to be the case. Uric acid is more likely a

modifier of the risk of cognitive decline in PD, one of many whose combination in the end is decisive in determining the rate of cognitive decline at the individual level.

The first submission of Study IV to the Journal on Neural Transmission also included data on uric acid, but the reviewers did not find this interesting and it was therefore excluded from the final publication.

The main strength of our studies is that patients had a good diagnostic accuracy concerning the diagnosis of PD and the evaluation was extensive with UPDRS and Hoehn and Yahr staging and both laboratory and neuropsychological testing. To date, Studies II and III are the only ones to examine the effect of uric acid on cognition in PD in a longitudinal setting with repeated neuropsychological testing. The laborious and expensive protocol explains the small sample size and also the lack of similar studies. Despite the small sample size, Study II found that low plasma and urine uric acid levels were associated with deterioration in the areas of cognition that are most vulnerable in PD and which often precede the development of PDD. That the plasma uric acid levels and cognition did not significantly change during follow-up may reflect the presence of several confounders in a small patient sample. At the end of the follow-up, it was not possible to reliably evaluate the prognostic value of plasma uric acid level on the rate of cognitive decline in PD. Our studies as well as others' point to low plasma uric acid level being a long-term condition predisposing to PD and faster progression of both motor and cognitive symptoms in PD. Whether the main determinant of plasma uric acid level is genetic, dietary or related to possible gastrointestinal pathology in PD remains unresolved.

Suggestions for future studies

The size of Study I was quite small, but allowed for a careful exclusion of the confounding factors. However, a larger study to verify the result is justifiable and might be possible with the advent of biobanks. The Helsinki Biobank could offer a plasma uric acid sample of all PD patients in the Hospital District of Helsinki and Uusimaa as well as samples of age- and gender-matched controls. This method would provide a large unselected sample of PD patients, although confounders, such as medication and dietary patterns, could not be evaluated.

To guide future studies on the postulated association between uric acid and cognition, I suggest that patients be recruited already at diagnosis. This would ensure that patients are at similar disease stages and more comparable and also would minimize changes in plasma uric levels during the course of the disease. The patient sample should be large enough to enable statistical analysis separately for males and females because the neuroprotective effect of uric acid may differ by gender. Also vascular risk factors should be evaluated, with laboratory testing of serum cholesterol and fasting glucose levels, history of hypertension and family history of cerebro- and cardiovascular diseases. Neuroimaging would also be mandatory to show existing vascular pathology.

A disease-modifying treatment, inosine, may become available in the near future. Prior to this, it would be important to gain more knowledge of uric acid's effect on cognition in PD. The fact that uric acid may be protective in some conditions and detrimental in others makes careful patient selection a critical feature in any treatment aiming to increase plasma uric acid levels.

Fortunately, one study has already been initiated in Spain called the Coppadis (Cohort of patients with Parkinson's disease in Spain 2015)[137]. This study aims to recruit 800 PD patients who will be followed for five years to examine the natural course and various aspects of the disease, including motor and non-motor symptoms, pain, neuropsychiatric symptoms, mood disorders and their impact on the quality of life of both patients and caregivers.

A subgroup of 300 patients will be selected to investigate also biomarkers, including serum uric acid and MRI of the brain. The study protocol does not include neuropsychological examination, but all patients will be tested with MMSE and Parkinson's disease cognitive rating scale and will have to complete a 16-piece puzzle. In my opinion, this study will provide key new data concerning the association of uric acid with cognition in PD.

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